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Role of Confined Water on the Compressibility Modulus of Lipid Monolayers
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The studies on lipid monolayer properties have been done by compressing the lipid spread in a large water surface up to its collapse. In this procedure, a surface pressure vs area per molecule isotherm can be obtained. In this methodology, it is assumed that the lipids spread at huge areas are considered as being in a gas phase and when they are compressed at a temperature below the critical one (the transition temperature) a condensed phase appears. The thermodynamic treatment is compared with a real gas in which the condensation is analyzed as a consequence of the manifestation of the intermolecular forces between the lipids. In other words, lipid interacts between them as gas particles in vacuum.
A more realistic approach to account on monolayer behavior is that lipids, even at large areas, are in contact with the water phase. Upon compression, the energy input is not merely used to make work of compression but to overcome the friction of hydrated lipid molecules with the water (stationary) phase. Thus, the lipids drag water during its compression, force its reorganization and/or a displacement work can occur. With this picture, the whole thermodynamic of lipid membrane is reevaluated. For this purpose, we analyzed the compression of the monolayer from a state identified as a free-force monolayer state up to the collapse for lipids with different hydration degrees (mainly PCs and PEs). The comparison of these compression curves with the branch of the isotherm obtained by compressing the lipids from the gaseous state allow to conclude that the water arrangements, mainly confined water and hydration water, determines the hysteresis and synergistic effects of membrane response.

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Adhesion-Induced Domain Formation in Multicomponent Membranes
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Lipid vesicles containing a relatively small number of molecular components provide simple model systems for cell membranes, which contain a large number of lipid and protein components. Such multicomponent membranes can undergo phase separation into two coexisting liquid phases and, thus, exhibit liquid domains within a liquid matrix. The associated phase diagrams depend on a number of parameters such as the overall membrane composition as well as on pH and temperature of the aqueous medium. Here, we address the influence of an adhesive substrate surface onto the phase behavior of the membranes. Specifically, we study giant unilamellar vesicles (GUVs) that can be prepared from different lipid mixtures and have sizes similar to those of cells. We control the adhesion by electrostatic membrane-surface interactions using a novel setup which allows tuning of the adhesion energy by an externally applied potential; see Fig. 1. With our single-vesicle approach, we can explore the phase behaviour of multicomponent membranes for different adhesion energies. One important objective is to identify distinct composition regimes for the phase separation in the bound and unbound part of the adhering vesicle membranes.
Figure -Side views (xz scans) of a one-component GUV (about 60 μm in diameter) obtained by confocal microscopy. Fluorescence of the GUV membrane is shown in green and the adhesion surface as a red line. Left: no applied potential. Right: 1V applied potential. The change in adhesion strength can be clearly seen.

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Intermembrane Forces and Membrane Deformation Observed via Dehydration and Osmotic Pressure
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Intermembrane interactions and forces that govern membrane structure can modulate lipid-protein interactions and thus affect cellular functions [1]. Here we address material properties of the membrane via structural deformation due to external stress using small-angle X-ray scattering (SAXS) and solid-state ^{2}H NMR spectroscopy. These techniques have been extensively used to study structural changes of membrane bilayer dispersions through application of osmotic pressure. However, distinguishing the effects of osmotic stress on intermembrane forces (separation force) versus membrane deformation requires further investigation [2]. We subjected model membranes (DMPC) in the liquid-crystalline state to dehydration and high osmotic pressures (up to 25 MPa). Using SAXS we were able to directly measure the interlamellar spacings and compare the results to solid-state ^{2}H NMR order parameters [1,3]. This approach allowed us to gauge the strength of intermembrane forces for a given hydration state and estimate the area per lipid and structural deformation at the molecular level. Under high osmotic pressure or low hydration we found large area deformations of up to 15% [1]. Also, we verified that the intermembrane force decays exponentially as a function of intermembrane distance as described by the hydration force theory [2]. However, temperature variation revealed decay constants of much larger than a single water molecule, possibly suggesting the existence of forces besides the hydration force. To provide insight into this we have introduced the osmotic coefficient (ratio of work of removing water to thermal energy) which distinguishes regimes of forces. These findings show significant area deformation of membranes and provide insight into the forces that govern intermembrane interactions. [1] K.J. Mallikarjunaiah *et al*. (2011) *BJ* **100**, 98-107. [2] V.A. Parsegian *et al.*. (1979) *PNAS***76**, 2750-2754 [3] H.I. Petrache and M.F. Brown (2007) *Meth. Mol. Biol*. **400**, 341-353.

1466-Pos Board B196
Phenomenological Elasticity Theory Approach to Bolalipid Membranes
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Extremophilic bacteria reveal exceptional stability and vitality under the high temperature, pressure and environment acidity. Such features are considerably provided by properties of their membranes composed of bolalipids. Therefore bolalipids appears to be very intriguing and promising object for the investigations. As opposed to ''conventional'' lipids bolalipids are composed of two polar head connected by two hydrocarbonic tails. As a rule they pierce through the whole membrane. At present the investigation of bolalipids is at its opening stage of the gathering of the various experimental and computer simulations data. However, for the effective progress in the investigation of the bolalipid membranes the elasticity theory should be developed. Similar theory applied to the conventional lipid membranes gives results that are in an excellent agreement with the experimental data. That is why we have chosen the way of the adaptation of the effective elasticity theory of lipid membranes to the case of bolalipids.
In the present work we constructed the phenomenological elasticity theory of the bolalipid membranes. The set of all feasible types of deformations are found and the number of the possible experiments for the determining of the elastic moduli are suggested. Theory takes into account the presence of the two configurations of bolalipids in the membrane: U-shape and O-shape configurations. The way of the experimental determination of the ratio of these configurations is proposed.

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Interaction of Phosphatidylinositol-4,5-Bisphosphate with Potential Clustering Agents Ca^{2+}, Mg^{2+}, and Cholesterol
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Phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] is an important signaling lipid in the cell plasma membrane, playing an important role in many diverse signaling processes. It is important to gain an understanding of how $PI(4,5)P_2$'s role in these signaling processes is regulated. For example, it has been proposed that regulation by $PI(4,5)P_2$ is based on its lateral localization within the plasma membrane, with multiple pools of $PI(4,5)P_2$ used for different signaling purposes. In vitro studies have indicated that both Ca^{2+} and cholesterol have the capacity to promote formation of $PI(4,5)P_2$ clusters in model membranes. To shed light on this we have examined the interaction of $PI(4,5)P_2$ with Ca^{2+}, Mg^{2+}, and cholesterol using solid-state MAS ^{31}P NMR. The solid state 31P-NMR allows us to examine the differential effects of Ca^{2+}, Mg^{2+}, and cholesterol on the 4 and 5 phosphomonesters of $PI(4,5)P_2$ independently. We examined phosphatidylcholine multilamellar vesicles containing near physiological concentration of $PI(4,5)P_2$ in the presence of micro and millimolar concentrations of Ca^{2+} and Mg^{2+}. The 4- and 5-phosphates of $PI(4,5)P_2$ were both found to shift downfield in the presence of Ca^{2+} and Mg^{2+}, indicating increased

deprotonation of PI(4,5)P_2 due to association of the cations. This effect was significantly larger in the presence of Ca^{2+}. Multilamellar vesicles containing PC and PI(4,5)P_2 with varying amounts of cholesterol were also studied. The 4- and 5-phosphates of PI(4,5)P_2 were found to have a significant downfield shift in the presence of 40 mol% cholesterol. The cumulative effects of cholesterol in combination with the common inner leaflet phospholipids, PE and PI, were also examined.

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Lipids as Regulators of Effective Membrane Rigidity

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The rigidity of lipid bilayer, the structural core of all cellular membranes, is one of the key force factor in membrane remodeling. For most of ''biomimetic'' lipid compositions the rigidity is thought to be only a weak function of the composition. However, for multicomponent membranes the composition is coupled to geometry, resulting in lateral redistribution of components in curvature gradients. Such redistribution can substantially facilitate local membrane deformations. We detected that such a decrease of apparent membrane rigidity in pure lipid bilayers containing physiological amounts of dioleoylphosphotidylethanolamin (DOPE), the lipid characterized by highly negative intrinsic curvature. Analyzing fast shape transformations of membrane nanotubes containing different amounts of DOPE we found that the membrane softening followed concentration-dependent redistribution of DOPE towards negative membrane curvature. The apparent bending rigidity of DOPE-containing membranes decreased almost twofold at 30mol% of DOPE, indicating that similar amount of DOPE in cellular membranes can substantially facilitate deformations.

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Enhancement in Lipid Bilayer Partitioning of Lysolipids and Fatty Acids Induced by their Composition

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Distribution of the solutes: lysopalmitoylphosphatidylcholine (LPPC), Palmitic acid (PA) and their 1:1 mixtures between water and dipalmitoylphosphatidylcholine (DPPC) bilayer were determined using a fluorescence probe that selectively detects these solutes in water. Membrane phase as well as solute concentration itself affects partitioning. Water solute concentrations were obtained at each of several bilayer lipid concentrations between 10 and 400 μM, from slopes of the linear variation of probe fluorescence properties with total solute concentrations of up to 10 % of the solvent lipid concentration. Dynamic Light Scattering experiments confirmed that the lipid/solute aggregates were vesicles in this range. Lipid concentration dependence of the solute component in water was fit to a thermodynamic model of solute distribution between two coexisting solvents. Water/bilayer partition coefficient and the solute transfer free energy were determined from the fit. Main findings are: (1) Water to bilayer transfer free energy of solute is lower for 0 to 2 % solute mole fraction than for 2 to 10 %, signaling composition induced bilayer relaxation that increases bilayer solubility, beginning at 2 % solute mole fraction. (2) Partition coefficients are in the order LPPC>PA> $LPPC^+PA$ at 37 °C. The enhanced partition coefficient of $LPPC^+PA$ signifies synergism toward increased solubility in the bilayer-gel phase. Enhancement effects were not present, where the DPPC bilayer is in the liquid phase, The observed order in the partition coefficients, at 50 °C, was LPPC≈$LPPC^+PA$>PA. The behavior of the partition coefficients in the gel and liquid phase is similar in character to the observed presence of synergism in the transmembrane permeabilityin the bilayer gel phase and lack of it in the liquid phase. The present results provide experimental evidence that increased presence of solutes in the membrane also enhance transmembrane permeability.

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A Comprehensive Study of Preferential Interaction of Cholesterol and its Fluorescent Analogs with Different Classes of Phospholipids

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Cell membranes are composed of various glycerophospholipids and sphingolipids. Cholesterol is believed to interact with these phospholipids to modulate the fluidity of the membrane. We have introduced a novel approach to measure the preferential interaction of cholesterol with different classes of phospholipids having different head-groups and acyl-chain compositions. Phospholipids involved in the study are phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM). These phospholipid classes constitute the bulk of the membrane phospholipids. The acyl chain configurations of the saturated and unsaturated glycerophospholipids involved in this study are di 16:0 and 16:0(*sn1*)-18:1(*sn2*) respectively. Correspondingly, the *N*-linked acyl chain configurations in SMs are 16:0 and 18:1(Δ9-cis). Affinity of sterol analogs cholestatrienol and bodipy-cholesterol towards phospholipid bilayers is also studied to compare their affinities with cholesterol. Large unilamellar vesicle (LUV) systems with (sterol) donor vesicles and (sterol) acceptor vesicles are used. Acceptor LUVs are allowed to accept the sterol from donor vesicles, transfer being assisted by methyl-β-cyclodextrin. Diphenylhexatriene-phosphatidylcholine (DPHPC) was used as a fluorescent probe in cholesterol donor acceptor anisotropy experiments. We observed that cholesterol showed different affinity for phospholipids as compared to its fluorescent analogs. Affinity of cholesterol and the fluorescent analogs for different phospholipid bilayers depended not only on the head groups of the phospholipids but also on their acyl chain configuration.

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Vesicles and Phase Dynamics: Cross-Linking Effects

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We study lipid phase behavior using giant unilamellar vesicles to model cell membrane dynamics. Vesicles allow us to isolate the lipid rearrangement due to cross-linking, common activity on cell surfaces. Biotylated lipids, avidin and its analogues allow us to model lipid rearrangement due to cross-linking at the headgroup position, where cross-linking is the linking of two molecules (biotinylated lipids) via a cross- linking agent (avidin). Using phase specific dyes, we study the changes that occur with the addition of a cross-linker to the system. Förster Resonance Energy Transfer (FRET) enables us to detect phase changes on the submicron scale, beyond the limits of conventional microscopy. Using FRET we detect lipid rearrangement associated with the transition from one-phase vesicles to two-phase vesicles using two different fluorescent dyes, a donor and acceptor. From this simple cross-linking system, we model membrane responses to protein complex formation and oligomerization.

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Domain Size Distribution in Phase Separated Cholesterol/Phospholipid Langmuir Monolayers: Line Tension and Transition Kinetics

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Multicomponent phase separated phospholipid monolayer systems of a canonical cholesterol and DMPC (~30/70 mole percent) have been used to study several aspects of cholesterol/phospholipid interaction and phase behavior. Despite the successful characterization and theoretical approaches applied to these systems there are still important details of monolayer morphology that are not fully understood. Here, we address the role of transition kinetics on domain size distributions. For our experiments, three different barrier speeds were chosen (4, 40, 400 cm squared per minute) as the monolayer passed through the miscibility phase transition (8.4 mN/m transition pressure). Average domain size was observed to decrease as the barrier speed increased (transition rate). The detailed size distribution measurements also provide the opportunity to measure changes in phase fraction and size distribution with monolayer surface pressures (2, 4, and 6, mN/m). Careful study of factors influencing the size distribution of phase separated domains is particularly relevant to the recently proposed line tension measurement technique (Lee et al., vol. 108 pp. 9425, PNAS 2011). We have implemented this method for the monolayer system studied here. A comparison to previously implemented line tension measurements based on a Fourier analysis of boundary fluctuations approaches will be presented. Finally, a brief comparison on the role of dyes and dye quality will be presented. Comparison among one year old Texas Red and new Texas Red, showed an impact in the sizes of domains.